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Alkaline Phosphatase Activity of Human Cell Cultures.* (26678)

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Studies of enzyme activities, nutritional requirements and metabolic activities associated with growth of mammalian cells in culture have in general revealed basically similar patterns regardless of the source from which those cells had been derived(1.2). During studies to characterize the tissue of origin of established human cell cultures, differences in alkaline phosphomonoesterase activity were observed(3). These observations have been extended to other cell lines, including recent isolates, and this report which describes differences in phosphatase activity between clonal strains of several parental lines, reemphasizes the heterogeneity which may exist in cell populations propagated *in vitro*.

Materials and methods. Established cell lines were purchased from Microbiological Associates, Inc., and clonal strains HeLa S_3 and 3213A were provided by Drs. T. T. Puck and M. Bender respectively. The parental Zimmer liver line was derived from a biopsy in a 5 month old infant with congenital atresia of the bile ducts and has been maintained in serial culture for approximately 70 passages. Recent cell isolates were derived according to standard technics from bone marrow aspiration(4) and skin biopsy(5). Clonal strains were derived from one or more consecutive single cell platings according to the procedure of Puck *et al.*(6).

Eagle's medium and M 199 containing 10 or 15% undialyzed human, horse or calf serum or the nutrient medium of Puck containing 20% undialyzed human serum were used for the growth of established cell lines. Recent isolates were cultured in an enriched Eagle's medium with added bovine embryo extract ultrafiltrate(4). After growth for 6-8 days on glass surfaces in an atmosphere of 5% CO_2 in air at 37°C, cells were dispersed with .05% trypsin in bicarbonate-free salt solution(7), and transferred to fresh medium, or harvested by centrifugation at 500 × g at room temperature after neutralization of tryptic action with growth medium. The cells were washed twice in 20 volumes of cold 0.14 M NaCl or 0.25 M sucrose and resuspended in 0.25 M sucrose. Cell-free extracts were prepared by sonic vibration of 2 to 5 ml of cell suspensions in collodion-treated lusteroid tubes for 1 to 5 minutes in an ice water-cooled Raytheon 10 kc oscillator.

Alkaline phosphatase was measured by hydrolysis of disodium p-nitrophenyl phosphate using 2-amino-2-methyl-1-propanol-HCl (AMP) buffer at pH 10.6 and 38°C(8). Specific activity was expressed as μM of p-nitrophenol liberated in 30 minutes per mg of cell protein(9).

Results. Table I summarizes the specific activities for alkaline phosphatase of a variety of established and recently isolated human cell lines. Each value represents the

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TABLE I. Alkaline Phosphatase Activity of Human Cell Cultures.

Established cell cultures	Specific activity*	Recent cell isolates	Specific activity
Detroit-6	170.7	Pe. marrow	90.9
J-111	127.2	As. marrow	29.3
Conjunctiva	106.0	Tu. skin	12.5
H.Ep. #2	96.5	He. marrow	8.7
Heart	51.2	Ch. skin	2.4
Chang liver	25.8		
KB	7.9		
Intestine	6.1		
Zi. liver	1.1		
HeLa S-3	0.3		

* Mean specific activity = μM p-nitrophenyl phosphate hydrolyzed per mg protein in 30 min. at 38°C in AMP buffer, pH 10.6.

mean of activities at termination of at least 4 consecutive 6-8 day growth cycles. There is a 500-fold difference between the phosphatase activities of established cell cultures; the highest values observed were those with the Detroit-6 and J-111 lines. Recent isolates from skin biopsy and bone marrow aspiration also showed a wide range of phosphatase activity even during the initial 4 or 5 passages *in vitro*. The variation in activity between different cell lines showed no relation to cellular morphology, nor did propagation in a particular nutrient medium explain the differences observed, since cell lines with high and low activity were found under the same growth conditions.

TABLE II. Changes in Alkaline Phosphatase Activity of Chang Liver during Serial Transfer Following Change of Nutrient Medium.

Days following medium alteration	Medium	Activity*
Initial	Eagle's + 10% horse serum	51.0†
7	Puck's + 20% human serum	54.8
19	*Idem*	14.9
25	"	8.4
33	"	11.4
40	"	12.1
47	"	15.6
55	"	39.0
77	"	70.1
99	"	72.3
107	"	87.4

* Specific activity = μM p-nitrophenyl phosphate hydrolyzed/mg protein in 30 min. at 38°C in AMP buffer, pH 10.6.

† Mean activity of cell preparations from 4 consecutive weekly growth cycles prior to transfer to new medium (individual values = 44.9, 41.0, 62.4, 55.7).

On the other hand, changes in alkaline phosphatase activity for a particular cell line were occasionally observed following alteration of the nutrient environment, as is shown in Table II with Chang liver cells following transfer from Eagle's medium containing horse serum to Puck's medium with human serum. This suggested the possibility that diverse cell types existed in the population, one or more of which might find a selective advantage in the new metabolic situation and so outgrow the other cells.

In support of this concept were the findings with clonal strains of various parental cell lines. Fig. 1 shows the distribution of

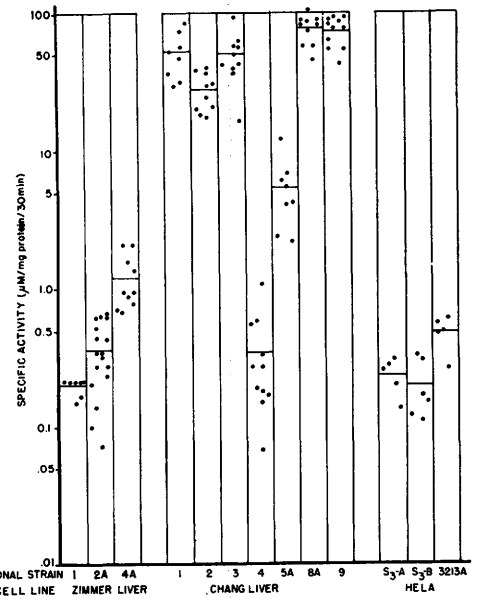

FIG. 1. Distribution of specific activities of alkaline phosphatase for clonal strains of various cell lines.

individual values and mean specific activities of alkaline phosphatase for clonal strains of Chang liver and Zimmer liver and subclones of HeLa S_3, during serial transfer in Puck's medium for 20 or more passages. There is a considerable difference in activity between the clonal strains. For example, Clone 4 of Chang liver, which can be distinguished from other Chang strains by the morphological characteristic of a smaller cross sectional area, has an average specific activity which is only 1/250th that of Clones 8A or 9. Similarly, a 6-fold difference in mean phospha-

tase activity is observed between Clone 1 and 4A of Zimmer liver, with no overlap between the maximum range of values. On the other hand, phosphatase activities of subclones A and B of HeLa S_3 are similar, and slightly lower than that of clonal strain HeLa 3213A.

Discussion. Although there is general uniformity in nutritional and metabolic characteristics, individual differences have been observed among certain cell lines in culture(1). The striking difference in alkaline phosphomonoesterase activity which has been noted also by Cox and MacLeod(10) is another such finding. Similar results have been obtained with histochemical technics(11,12,13) although these methods do not lend themselves to precise quantitation. Kovacs also has reported differences in phosphatase activity as measured at pH 7.6 with a biochemical assay using intact cell cultures(14).

The phosphatase activity of sonicates of a given cell line remains constant during serial propagation under uniform environmental conditions. However, the variability occasionally observed following transfer to a different nutrient medium suggested the existence of diverse cell types in the population, one or more of which might find a selective advantage as a result of the new metabolic situation and so outgrow the other cells. Studies with clonal cell strains derived from several parental lines showed significant differences in alkaline phosphatase activity, in support of this concept. In fact, the maximum range of activity with clonal isolates from a single parental line was almost as great as the range of activity with different parental lines. These differences are in agreement with the findings that clonal strains may have distinct physiological and morphological properties as well as observable differences in their karyotype(15,16). Since the available evidence suggests that the phenotypic variability of aneuploid cell populations may be caused by changes in the chromosomal constitutions of cells(16) it will be of interest to determine whether alterations of karyotype can be correlated with differences in alkaline phosphatase activity of various clonal strains.

Summary. Studies of alkaline phosphatase activity of sonicates of human cell cultures have revealed striking quantitative differences between established and recently isolated cell lines of diverse origin. Marked differences also have been noted between clonal strains derived from one or more consecutive single cell platings from several cell lines. The changes in phosphatase activity during serial passage following alteration of the nutrient medium may have resulted from a change in the relative distribution of cell types within the parental population.

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